

Reconstructing Roma history from genome-wide data

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Abstract

The Roma people, living throughout Europe, are a diverse population linked by the Romani language and culture. Previous linguistic and genetic studies have suggested that the Roma migrated into Europe from South Asia about 1,000-1,500 years ago. Genetic inferences about Roma history have mostly focused on the Y chromosome and mitochondrial DNA. To explore what additional information can be learned from genome-wide data, we analyzed data from six Roma groups that we genotyped at hundreds of thousands of single nucleotide polymorphisms (SNPs). We estimate that the Roma harbor about 80% West Eurasian ancestry—deriving from a combination of European and South Asian sources—and that the date of admixture of South Asian and European ancestry was about 850 years ago. We provide evidence for Eastern Europe being a major source of European ancestry, and North-west India being a major source of the South Asian ancestry in the Roma. By computing allele sharing as a measure of linkage disequilibrium, we estimate that the migration of Roma out of the Indian

subcontinent was accompanied by a severe founder event, which we hypothesize was followed by a major demographic expansion once the population arrived in Europe.

Authors Summary

Inferences of history based on autosomal genetic markers can provide precise information about a population's history. To characterize the history of the Roma gypsy population, we applied genomic methods based on allele frequency correlations, linkage disequilibrium, and identity-by-descent sharing. We provide formal evidence that the Roma have ancestry from West Eurasians and South Asians, with the likely sources related to Eastern Europeans and North-west Indians respectively. We estimate that the major gene exchange occurred about 850 years ago, soon after the exodus of Roma out of the Indian sub-continent. The migration out of India was accompanied by a severe founder event, signatures of which have been preserved for hundreds of years because of the endogamy prevalent in the Roma community.

Introduction

The Roma (also called Romani or Gypsies) represent a unique and diverse European population. They speak more than 60 dialects of a rapidly evolving language called *Romani* and belong to different social and religious groups across Europe. Their census size has been estimated to be in the range of 10-15 million[1], with the largest populations in Eastern Europe[2]. They do not have written history or genealogy (as Romani does not have a single convention for writing) and thus most of the information about their history has been inferred based on linguistics, genetics and historical records of the countries where they have resided.

Previous studies have suggested that the Roma are originally from India, and that they migrated to Europe between the 5th and 10th century[3]. It has been argued that their migration route included Persia, Armenia, Anatolia and Greece[3,4]. The Roma then settled in multiple locations within Europe and descendants of these migrants mostly live in the Balkans, Spain and Portugal today. By the 15th century, the Roma were present in almost all parts of Europe[5].

Anthropological studies of the Roma have documented striking similarities between the cultures of various Indian groups and Roma. Social structure in Roma groups is similar to the *castes* of India, where the groups are often defined by profession[2,3]. Like many Indian populations, the Roma practice endogamy and individuals of one Roma clan (sub-ethnic group) preferentially marry within the same group, and marriages across clans are proscribed[3]. Many studies have also suggested a link between the Roma and *Banjara* (the wandering gypsy tribes of India) currently residing in central and southern India[3]. Linguistic analysis of the Banjari or Lamani, languages spoken by the Indian gypsies, have little similarity to Romani[6]. Linguistic and genetic studies, however, have provided strong evidence for the origin of Roma in India. Y-chromosome marker H1a-M82 and mitochondrial haplogroup M35b, both thought to be characteristic of South Asian ancestry, are present at high frequency in Roma populations[7,8].

These studies have also documented that since their migration into Europe, the Roma have admixed with neighboring European and West Asian populations[7,8]. However, there is no consensus about the specific ancestral group/ geographic region within South Asia that is related to the ancestral population of the Roma. Comparative linguistics suggests that Northwestern Indian languages like Punjabi or Kashmiri or Central Indian languages like Hindi are most closely related to Romani[9,10]. A recent study based on Y-chromosome markers suggests that the Roma descended from Southern Indian groups[11], which is contradictory to previous reports based on mtDNA haplogroups that have placed the origin of Roma in North-west India. While mtDNA and Y chromosome analyses provide valuable information about the maternal and paternal lineages, a limitation of these studies is that they represent only one instantiation of the genealogical process. Autosomal data permits simultaneous analysis of multiple lineages, which can provide novel information about population history.

Here we have analyzed whole genome SNP array data from 27 Roma samples belonging to six groups that were sampled from 4 countries in Europe (three separate ethnic groups from Hungary, and one group each from Romania, Spain and Slovakia). Our aim was to address the following questions: (1) What is the source of the European and South Asian ancestry in the Roma? (2) What is the relationship of the Roma to the present-day South Asian populations? (3) Do present-day Punjabis or South Indians best represent the ancestral South Asian component of Roma? (4) What is the proportion and timing of the European gene flow? (5) Can we identify founder events or detect genetic signatures of endogamy?

Results

Genome-wide ancestry analysis of the Roma

We applied Principal Component Analysis (PCA) using the SMARTPCA software[12] and the clustering algorithm ADMIXTURE[13] to study the

relationship of Roma to other worldwide populations in a merged dataset of Roma and HapMap3 populations. In PCA, the Roma fall between the South Asians (Gujaratis) and Europeans, consistent with their having both South Asian and European ancestry and in line with previous mtDNA and Y chromosome analyses[7,8] (Figure 1). The ADMIXTURE software, which implements a maximum likelihood method to infer the genetic ancestry of each individual modeled as a mixture of K ancestral groups, produces very similar inferences[13]. The resulting clustering plot for K=6 is shown in Figure 1 and for other K values in Figure S1. At K=6, we observe clustering based on major continental ancestry. Similar to the PCA results, the Roma individuals cluster with South Asians and Europeans (Figure 1). Based on the PCA and ADMIXTURE analysis, we excluded three Roma outlier samples from further analyses, as they appeared to have very recent admixture from neighboring non-Roma European populations (likely in the past few generations). We also examined pairwise average allele frequency differentiation (F_{st}) between Roma and major continental groups (see Table S1).

Previous studies have shown that most present-day South Asians populations trace their ancestry to major ancestry components- one related to West Eurasians (referred to as Ancestral North Indians (ANI)) and the other related to indigenous Andamanese population (Onge) (Ancestral South Indian (ASI))[14]. This mixture is pervasive in South Asia and signatures of this mixture are present in all caste and social groups and in speakers of Indo-European and Dravidian languages[15]. As the Roma trace their ancestry to similar ancestral populations, we performed PCA to study the relationship of Roma with the present-day South Asians and HapMap populations. We observed that like all South Asians, the Roma also fall on the “Indian-cline” (which refers to the differential pattern of relatedness of South Asians to Europeans). However, they have much higher proportion of European ancestry compared to any other South Asian group (Figure 1c).

We performed a *4 Population Test*[15] to test formally if the Roma have evidence of a mixture of European and South Asian ancestry. We used individuals of Northern European ancestry (CEU) and Andamanese as surrogates for the European and South Asian ancestral populations. We tested whether the phylogenetic tree (Africans, Europeans, South Asians, Roma) is consistent with the data. We choose Onge for this analysis, since, unlike their distant relatives on the Indian mainland, they do not have any West Eurasian related admixture[15]. Applying the *4 Population Test* to each of three simple phylogenetic trees that could potentially relate the four groups, we observed highly significant violations of the expected phylogenetic tree topology, confirming that the Roma are admixed; that is, they have ancestry from both South Asians and Europeans (Table S2). We note that this test does not distinguish between European and West Asian ancestry and hence we refer to this ancestry component as West Eurasia ancestry.

To quantify the magnitude of the South Asian and West Eurasian ancestry in the Roma, we applied *f₄ Ratio Estimation*[15], which can estimate admixture proportions in the absence of data from accurate ancestral populations. This test estimates the excess of West Eurasian-related ancestry in Roma compared to an Onge (who have no known West Eurasian related ancestry[16]). Applying the *f₄ Ratio Estimation* to Roma assuming the tree shown in Figure S2, we estimate that the Roma have $77.5 \pm 1.8\%$ West Eurasian related ancestry (standard errors were computed using a Block Jackknife with a block size of 5cM) (Table S2). We note that some of the West Eurasian related ancestry we detect likely derives from India itself—from the ANI—while other parts may derive from a European mixture (post exodus from India).

Estimating a date of European admixture in the Roma

To estimate the timing of the admixture event, we applied a modified version of *ROLLOFF*[17], which uses the decay of admixture linkage disequilibrium (LD) to estimate the time of gene flow. *ROLLOFF* computes SNP correlations in the

admixed population and weights the correlations by the allele frequency difference in the ancestral populations such that the signal is sensitive to admixture LD. While this method estimates accurate dates of admixture in most cases, we observed that it is noticeably biased in case of strong founder events post admixture (Table S3). The bias is related to a normalization term that exhibits an exponential decay behavior in the presence of a strong founder event, thus confounding the admixture date (see details in Note S1, Figure S3). We propose a modification to the *ROLLOFF* statistic that removes the bias (Note S1, Table S3). In addition, the new statistic computes covariance instead of correlation between SNPs; this does not affect the performance of the method but makes it mathematically more tractable. Throughout the manuscript, we use the modified *ROLLOFF* statistic ($R(d)$) unless specified otherwise. Simulations show that this statistic gives accurate and unbiased results up to 300 generations (Note S2, Figure S4).

A feature of our method is that it uses allele frequency information in the ancestral populations to amplify the admixture signal relative to background LD. While data from the ancestral populations is not available for Roma, this information can be obtained by performing PCA using the present day Europeans and South Asians. Simulations show using PCA-based SNP loadings effectively capture the allele frequency differentiation between the ancestral populations and can be used for estimating dates of mixture (Note S2, Figure S5).

Applying the modified *ROLLOFF* statistic to the Roma samples with the SNP loadings estimated using PCA of Europeans (CEU) and 16 Indian groups, we estimate that the West Eurasian admixture in Roma occurred 29 ± 2 generations or about 780-900 years ago in the past assuming one generation = 29 years[18] (Figure 2). This is consistent with mixture having occurred only after the historically recorded arrival of the Roma in Europe between 1,000-1,500 years ago[3]. A potential complication is that the date we are estimating may also be reflecting earlier admixture of ANI and ASI ancestry in India itself. However,

when we fit the decay of admixture LD using two exponential distributions to accommodate the possibility, we obtain dates of 37 and 4 generations. The older date corresponds to about 1,000 years before present – again consistent with the historical record – and both dates are much more recent than any estimates obtained by applying *ROLLOFF* in India. This suggests that the admixture we are detecting is genuinely related to events in Europe.

Relationship with the host European populations

To learn about the relationship of the Roma with neighboring European populations, we estimated the pairwise Identity-by-descent (IBD) sharing between each Roma individual and non-Roma individuals sampled from the respective countries (Slovakia ($n = 1$), Romania ($n = 14$), Hungary ($n = 19$) and Spain ($n = 137$)). IBD segments (>3 centimorgans (cM)) were detected using GERMLINE[19]. The output of GERMLINE was used to compute an average pairwise sharing distance between Roma from each geographic region and the host populations from that region (Figure S6). We observe that Roma exhibit the highest IBD sharing with individuals from Romania (Figure 3a). When we perform stratified analysis (where each Roma group is considered separately), we observe that the highest sharing is with Romania or Slovakia, consistent with the hypothesis that the admixture involved populations in Eastern Europe. However, we have very limited samples from some populations here, hence it would be important to repeat this analysis with more samples.

Source of the South Asian ancestry in Roma

To learn about the source of the South Asian ancestry in Roma, we inferred the pairwise IBD sharing distance between Roma and various Indian groups, using GERMLINE to compute an average pairwise sharing distance between Roma and 28 South Asian populations (24 Indian groups from the India Project, Pathan and Sindhi from HGDP and Punjabi and Gujarati from POPRES). To simplify the analysis, we classified the samples into 8 groups based on geographical regions

within India: North ($n = 38$), Northwest ($n = 235$), Northeast ($n = 8$), Southwest ($n = 16$), Southeast ($n = 59$), East ($n = 11$), West ($n = 42$) and Andamanese ($n = 16$). We observed that the Roma share the highest proportion of IBD segments with groups from the Northwest (Figure 3b). Interestingly, the two populations in our sample that show the highest relatedness to Roma (Punjabi, Kashmiri Pandit) are also the populations that have highest proportion of West Eurasian-related (ANI) ancestry. To control for the possibility that the high IBD sharing could be an artifact related to high ANI ancestry, we recalculated the IBD sharing regressing out the ANI ancestry proportion and observed that the Roma continue to share the highest IBD segments with the northwest Indian group (Note S3). These findings are consistent with analyses of mtDNA that also place the most likely South Asian source of the Roma in Northwest India[8].

An important caveat is that we have large discrepancy in the number of samples available from different regions in India. In order to control for the sample sizes, we performed bootstrap analysis drawing a random sample up to 30 individuals from each Indian group and recomputing the IBD statistics. We repeated the process a 100 times and estimated the mean and standard error. We observed that Roma still have the highest IBD segments with Northwest Indian groups. However, it is interesting to note that there is very little variability across the 100 runs, suggesting that we are perhaps picking up shared signals of selection or founder events between Roma and Indian groups (Note S3, Figure S7).

Characterizing the founder events

Previous genetic and social studies have shown that the present day Roma population has descended from a small number of ancestors with subsequent genetic and cultural isolation[8,20]. A history of founder events in a population can lead to an increase in homozygosity and large stretches of allele sharing across individuals within the same population. This can be measured by estimating the proportion of the autosomal genome that has homozygous genotypes. We applied PLINK v1.07[21] to compute a genomic measure of

individual autozygosity for all Roma individuals and 30 random individuals from each HapMap population. PLINK uses a sliding window approach to find regions of the genome that are at least 1MB in length and contains 100 contiguous homozygous SNPs. For each individual, we computed the overall length of the autozygous segments and observed that the Roma have very high level of autozygosity compared to other HapMap populations (Figure 4a).

To estimate the date of the founder event in Roma, we computed a distance based statistic that measures allele sharing as reported in Reich et al (2009)[16]. This method is based on computing the autocorrelation of allele sharing between pairs of individuals from one group, and then subtracting the cross-population autocorrelation to remove the effects of ancestral allele sharing inherited from the common ancestor. By measuring the exponential decay of auto-correlation with genetic distance, we obtain an estimate of the age of the founder event. Simulations have shown that this method can accurately estimate the dates of recent founder events even in the presence of admixture (Note S4).

Applying this method to Roma and subtracting the shared Roma and European (CEU) autocorrelation, we estimate that a Roma founder event occurred 27 generations or ~800 years ago (assuming one generation = 29 years[18]) (Figure 4b). This is consistent with reports that the Roma exodus from India occurred 1,000 years ago[3], and suggests that the migration out of the Indian sub-continent may have been associated with a significant founder event in which a small number of ancestral individuals gave rise to the present-day Roma population.

Discussion

Using genome-wide SNP data from 27 Roma individuals, we have provided (1) confirmation of previous mtDNA and Y chromosome results with autosomal data, and (2) some new insights that take special advantage of autosomal data.

We have performed formal tests to confirm that Roma are admixed and have ancestry from two highly divergent populations: a West Eurasian population and a South Asian population. We estimate that the Roma have ~80% West Eurasian ancestry, reflecting a combined estimate of the ANI ancestry that the Roma derive from their South Asian ancestors (pre-exodus) and the European ancestry related to the admixture in Europe (post-exodus to Europe). Our estimate is broadly consistent with admixture proportions estimated using autosomal short tandem repeats (66-100%)[22]. We only estimate a combined estimate for the West Eurasian ancestry and so our estimate of $76 \pm 4\%$ West Eurasian ancestry in the Spanish Roma is not discrepant with the estimates of European ancestry (post-exodus only) of 30% based on mtDNA markers and 37% based on Y chromosome markers reported previously[8,23].

Our identity-by-descent analysis provides novel insights related to the source of the ancestral populations of Roma. We provide evidence for Eastern Europe being a major source of European ancestry, and North-west India being a major source of the South Asian ancestry in the Roma. Our inferences about the geographic origin within South Asia help resolve a long- standing debate related to the origin of the Romani people. Our results are consistent with reports from linguistics[9] and mtDNA studies[8], which have shown that present day Northwest Indian populations (from Kashmir and Punjab), are good candidates for being the source of the Indian ancestry in Roma[8,23]. However, we caution that IBD based methods require large sample sizes from the source and target populations. Hence, a larger sample size will increase the power to detect subtle differences between geographic regions.

A historically informative insight from our analysis is the date of the West Eurasian gene flow into Roma. Using a statistic that captures the pattern of

admixture related linkage disequilibrium, we estimate that the admixture between Roma and West Eurasians occurred 29 ± 2 generations or about 780-900 years ago (assuming one generation = 29 years[18]). The earliest records of the arrival of Roma in the Balkans date back to the 11th-12th century[3], which is concordant with our estimated date of mixture[3]. It is important to note that the Roma have ancestry from both ANI and Europeans and thus the estimated date of admixture with Europeans (post exodus) is slightly downward biased (older). Simulations have shown in the case of two gene flow events, the date of admixture estimated by *ROLLOFF* tends to reflect the date of the recent gene flow event, if the interval between the two events is sufficiently large (Table S4, Note S2).

Disease mutation screening in the Roma has shown that they have an increased proportion of private mutations[20]. For example, deletion 1267delG that causes a neuromuscular disorder, *congenital myasthenia*, has a high carrier frequency in many Roma groups that reside in different parts of Europe and speak different languages. In addition to the Roma groups, this mutation has only been observed in South Asian populations before[20,24]. This provides evidence that the different Roma groups have a history of a shared founder event. In order to obtain temporal information of the founder event that has likely increased the frequency of such disease causing mutations, we studied LD based allele sharing statistics and estimated that the founder event in Roma occurred about 27 generations, or 800 years, ago. This agrees with previous reports from Morar et al. (2004)[24] who hypothesize that the entire Roma population was founded about 32-40 generations ago.

Our results have confirmed that the Roma have ancestry from South Asians and West Eurasian populations, with mixture occurring around 30 generations ago. An important opportunity for future work is to perform homozygosity mapping in Roma that can aid in finding disease-causing mutations related to the founder events. In addition, it would be illuminating to study the relationship of the Roma with other gypsy populations especially the *Banjara* from India. This may provide new insights into the history of Roma and perhaps help to elucidate the historical reasons for their exodus.

Materials and Methods

Datasets: We collected 27 Roma samples belonging to six groups that were sampled from four countries in Europe from Hungary (3 linguistically and culturally separated sub-groups: 7 samples from Olah (Vlah), 4 samples from Beas (Boyash) and 4 samples from Romungro)); 4 samples from Romania, 4 samples from Spain and 4 samples from Slovakia (Slovakian speaking Roma)). All research involving human participants was approved by the Regional Ethics Committee Board (REKEB) and the Hungarian National Ethics Committee (ETT TUKEB). Each study participant attended a 45-60mins verbal orientation session about the study design and goals and then provided written informed consent. All the research was conducted according to the principles expressed in the Declaration of Helsinki. Roma individuals self-reported as being descendants of the same tribe for at least three generations. The samples were genotyped using an Affymetrix 1M SNP chip. We required < 5% missing genotype rate per sample per SNP to be included in the analysis (27 individuals, 726,404 SNPs passed this threshold). These data were merged with data from four other sources, including the International Haplotype Map Phase 3 (HapMap3) ($n=1,115$ samples from 11 populations genotyped on Affymetrix 1M array)[25], the CEPH-Human Genome Diversity Panel (HGDP) ($n = 257$ individuals from 51 populations genotyped on Affymetrix 500K SNP array)[26,27], our previous study of Indian genetic variation which we call the “India Project” in this paper ($n = 132$ individuals from 25 groups genotyped on an Affymetrix 1M SNP array)[15] and the Population Reference Sample (POPRES) ($n = 3,845$ individuals from 37 European populations genotyped on an Affymetrix 500K SNP array)[28].

Population Structure Analysis and F_{st} calculation: We created a merged dataset of Roma and HapMap3 populations ($n = 1,142$ and 853,727 SNPs). As background LD can affect both PCA and ADMIXTURE analysis, we thinned the marker set by excluding SNPs in strong LD (pairwise genotypic correlation $r^2 > 0.1$) in a window of 50 SNPs (sliding the window by 5 SNPs at a time) using PLINK v1.07[21]. The thinned dataset contained 61,052 SNPs. We used

SMARTPCA[12] to carry out PCA and to compute F_{ST} values. Clustering analysis was performed using ADMIXTURE[13].

Formal tests of population mixture: To test if Roma have West Eurasian and Indian ancestry, we used the unrooted phylogenetic tree ((YRI, CEU), (Onge, Roma)) and computed the *4-population test* statistic for all three phylogenetic trees that can possibly relate these populations. For this analysis, we created a merged dataset of Roma, India project and HapMap3 populations ($n = 1,274$ and $524,053$ SNPs). Let YRI_i , CEU_i , $Onge_i$ and $Roma_i$ be the allele frequencies for SNP i in the populations YRI, CEU, Onge and Roma respectively. Then we compute the $\rho(YRI_i-CEU_i, Onge_i-Roma_i)$ for all SNPs across the genome. In the absence of mixture, we would expect this correlation to be almost 0. Standard errors were computed using Block Jackknife[29,30] where a block of 5cM was dropped in each run.

Estimating genome-wide ancestry proportion: We estimate the genome-wide proportion of ancestry using *f_4 Ratio Estimation*[15] which estimates the excess of European ancestry compared to an Onge. We use the phylogenetic tree (YRI,(CEU,(Adygei,(Onge,Roma)))) as shown in the Figure S2. It has been shown previously that ANI form a clade with CEU and Onge form a clade with ASI[15]. YRI and Adygei are used as outgroups in this analysis. Let YRI_i , CEU_i , $Adygei_i$, $Onge_i$ and $Roma_i$ be the allele frequencies for SNP i in the populations YRI, CEU, Adygei, Onge and Roma respectively. We compute ratio of the $f_4(YRI_i, Adygei_i; Roma_i-Onge_i) / f_4(YRI_i, Adygei_i; CEU_i-Onge_i)$. This quantity is summed over all markers and the standard errors are computed using the Block Jackknife (block size of 5cM). To represent all the populations needed for this analysis, we created a merged dataset that included data from Roma, India project, HGDP and HapMap3 ($n = 1,531$ and $262,558$ SNPs).

GERMLINE analysis: IBD segments were detected using GERMLINE[19]. For this analysis, we phased the data using Beagle[31] and then ran GERMLINE in Genotype Extension mode on a combined dataset of Roma, HapMap3, India

Project, POPRES and HGDP ($n = 5,376$ and $205,710$ SNPs). We applied the following parameters for calculating IBD segments: seed size = 75, minimum IBD segments length = 3cM long and the number of heterozygous or homozygous errors = 0. The output of GERMLINE was used to compute an average pairwise sharing between populations I and J as previously reported in reference [32].

$$\text{Average sharing} = \frac{\sum_{i=1}^n \sum_{j=1}^m IBD_{ij}}{n \times m}$$

where IBD_{ij} = the length of IBD segment shared between individual i and j and n, m are the number of individuals in population I and J .

Estimation of a date of mixture: We applied modified *ROLLOFF*[17] to estimate the date of mixture in a combined dataset containing 1,274 individuals and 524,053 SNPs. For each pair of SNPs (x, y) separated by a distance d Morgans, we compute covariance between (x, y). Specifically, we use the following statistic -

$$R(d) = \frac{\sum_{|x-y|=d} z(x, y)w(x, y)}{\sum_{|x, y|=d} w(x, y)^2}$$

where $z(x, y)$ = covariance between SNPs (x, y) and weight function $w(x, y)$ = weight function that can be the allele frequency difference between the ancestral populations or the PCA based loadings for SNPs (x, y). We look at the relationship of the weighted covariance with genetic distance, and obtain a date by fitting an exponential function with an affine term $y = Ae^{-nd} + c$, where n is the number of generations since admixture and d is the distance in Morgans. Standard errors were computed using a Block Jackknife[29,30] where one chromosome was dropped in each run.

Estimating individual autozygosity: We used PLINK v1.07[21] to identify autozygous segments in the genome in a combined dataset of 1,274 individuals and 524,053 SNPs. PLINK uses a sliding window approach to find regions of the

genome that are at least 1MB in length and contains 100 contiguous homozygous SNPs. We allowed one heterozygous and five missing calls per segment. Autozygous segments were identified separately for each individual. We computed the overall length of autozygous segments for each individual as a their measure of genomic autozygosity. We applied this method to compute genomic autozygosity for Roma and HapMap individuals ($n = 30$ from each population).

Estimating a date of founder event: To estimate the date of the founder event, we compute the correlation of allele sharing as a measure of LD as described in reference [15] using a dataset containing Roma and HapMap3 populations ($n = 1,142$ and $853,727$ SNPs). Specifically, we compute the autocorrelation of allele sharing between pairs of individuals of one group, and then subtract the across-population autocorrelation to remove the effects of ancestral allele sharing. We thus get a measure for the population-specific LD. We plot the auto-correlation with genetic distance and by fitting the exponential function $y = Ae^{-2tD} + c$, where $D =$ distance in Morgans and $t =$ time of founder event, we estimate the age of the founder event.

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Figure Legends

Figure 1. Relationship of Roma with other worldwide populations. We applied Principal Component Analysis (PCA) and the clustering algorithm ADMIXTURE to study the relationship of Roma with the HapMap3 and South Asian populations. Panel (a) shows the results for PCA of Roma and HapMap3 populations where each point represents an individual and the coloring is based on the legend shown on the right. Panel (b) shows the results for ADMIXTURE for K=6 for Roma and HapMap3 populations. Each vertical line represents an individual colored in proportion to their estimated ancestry within each cluster. Panel (c) shows the results of PCA of Roma, HapMap3 (CEU, CHB) and South Asian populations. The populations codes are as follows: Yoruba in Ibadan (YRI), Nigeria, Luhya in Webuye, Kenya (LWK), Maasai in Kinyawa, Kenya (MKK), Utah residents with Northern and Western European ancestry (CEU), Toscani in Italia (TSI), Han Chinese in Beijing, China (CHB), Japanese in Tokyo, Japan (JPT), Chinese in Metropolitan Denver, Colorado (CHD), Gujarati Indians in Houston, Texas (GIH), African ancestry in Southwest USA (ASW) and Mexican ancestry in Los Angeles, California (MEX).

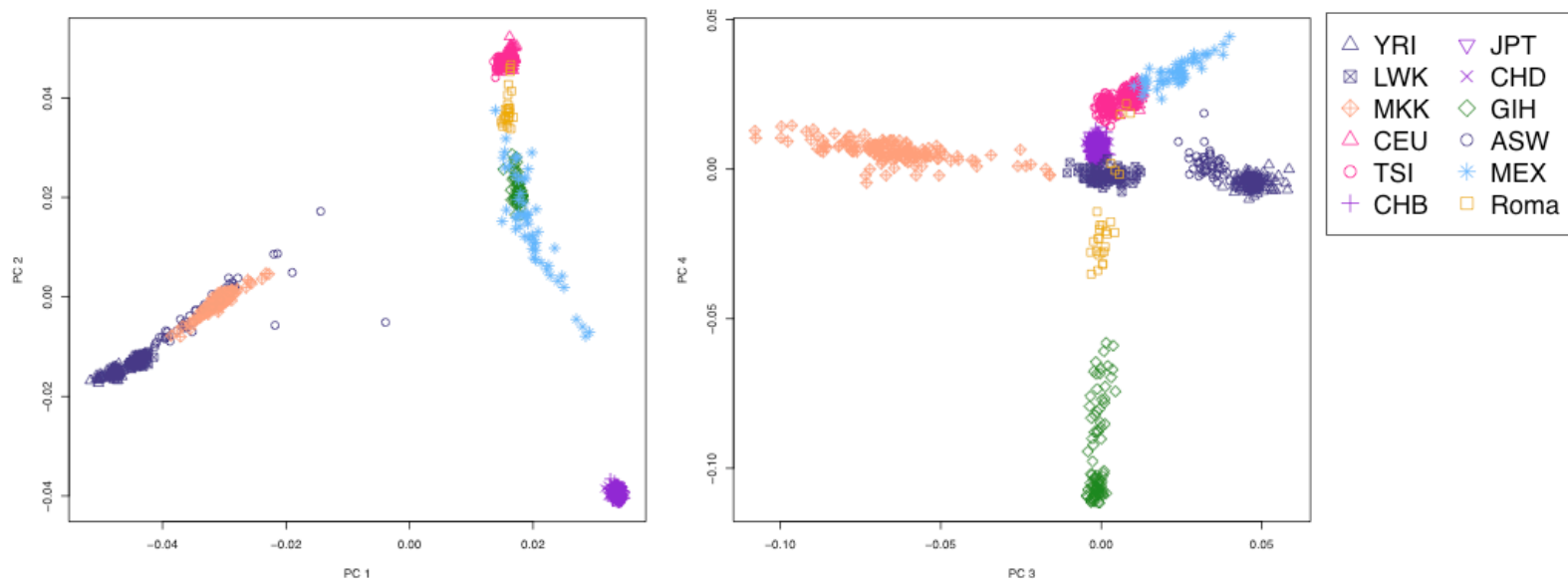
Figure 2. ROLLOFF Analysis of Roma. We performed *ROLLOFF* on the Roma samples (# samples = 24). We plot the weighted covariance as a function of genetic distance, and obtain a date by fitting an exponential function with an affine term $y = Ae^{-nd} + c$, where d is the genetic distance in Morgans and n is the number of generations since mixture. We do not show inter-SNP intervals of <0.5cM since we have found that at this distance admixture LD begins to be confounded by background LD.

Figure 3. Evidence for the European and South Asian sources of Roma ancestry. We computed a genome-wide average IBD sharing distance between Roma and other populations. Panel (a) shows average pairwise IBD sharing between Roma and Europeans (non-Roma European individuals from the countries in which the Roma were sampled) and panel. All Roma samples were combined in one group and (b) shows IBD sharing average pairwise IBD sharing

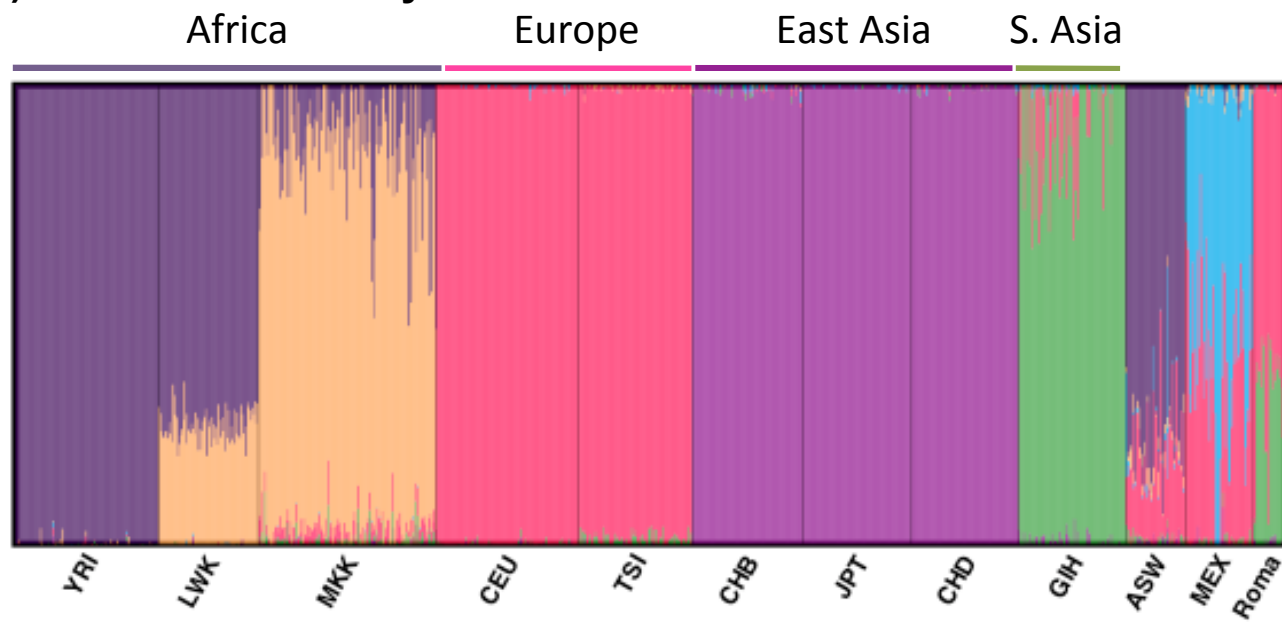
between Roma and individuals from India. Indians were grouped in seven regional categories as follows: *North* includes Tharu, Kharia, Vaish, Srivastava, Sahariya, Lodi, Pathan and Sindhi, *Northwest* includes Kashmiri Pandit and Punjabi, *Northeast* includes Nyasha and Ao Naga, *Southwest* includes Kurumba and Hallaki, *Southeast* includes Madiga, Mala, Vysya, Chenchu, Naidu, Velama and Kamsali, *West* includes Bhil, Meghawal and Gujarat, *East* includes Santhal and Satnami and *Andamanese* includes Great Andamanese and Onge. Detailed description of these populations can be found in reference [15].

Figure 4. Inferring founder events in the Roma. Panel (a) shows estimates of genomewide autozygosity in Roma and individuals from HapMap (n = 30 from each population). Each point represents an individual colored based on the legend shown below. Panel (b) shows the decay of autocorrelation with genetic distance. We fitted an exponential function $y = Ae^{-2tD} + c$ where D = distance in Morgans and t = time of founder event to estimate the time of founder event(s) as 27 generations.

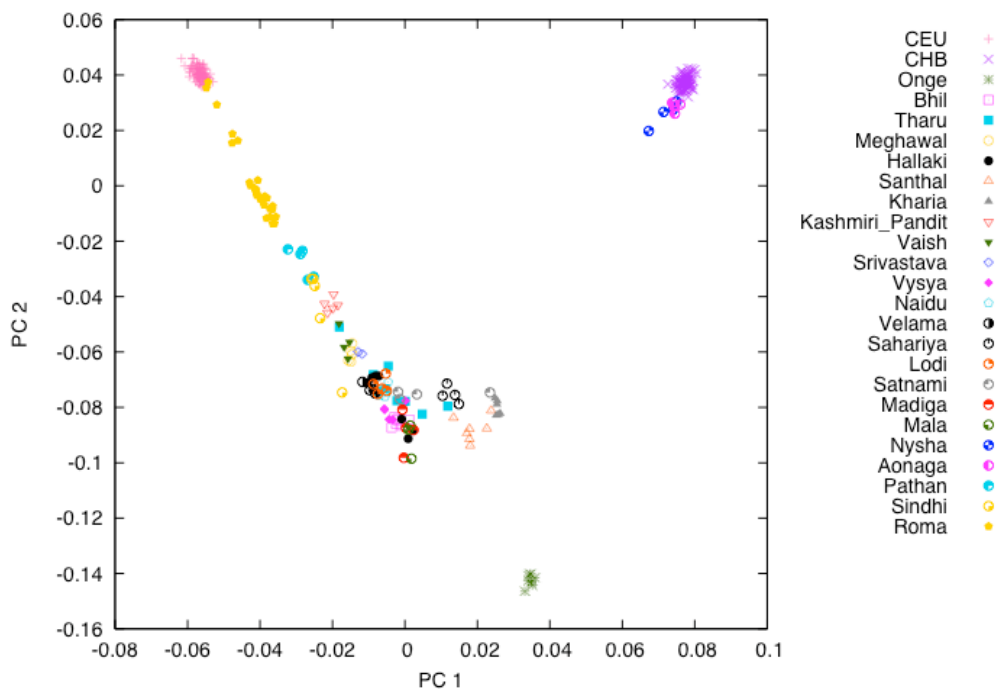
(a) Principal Component Analysis



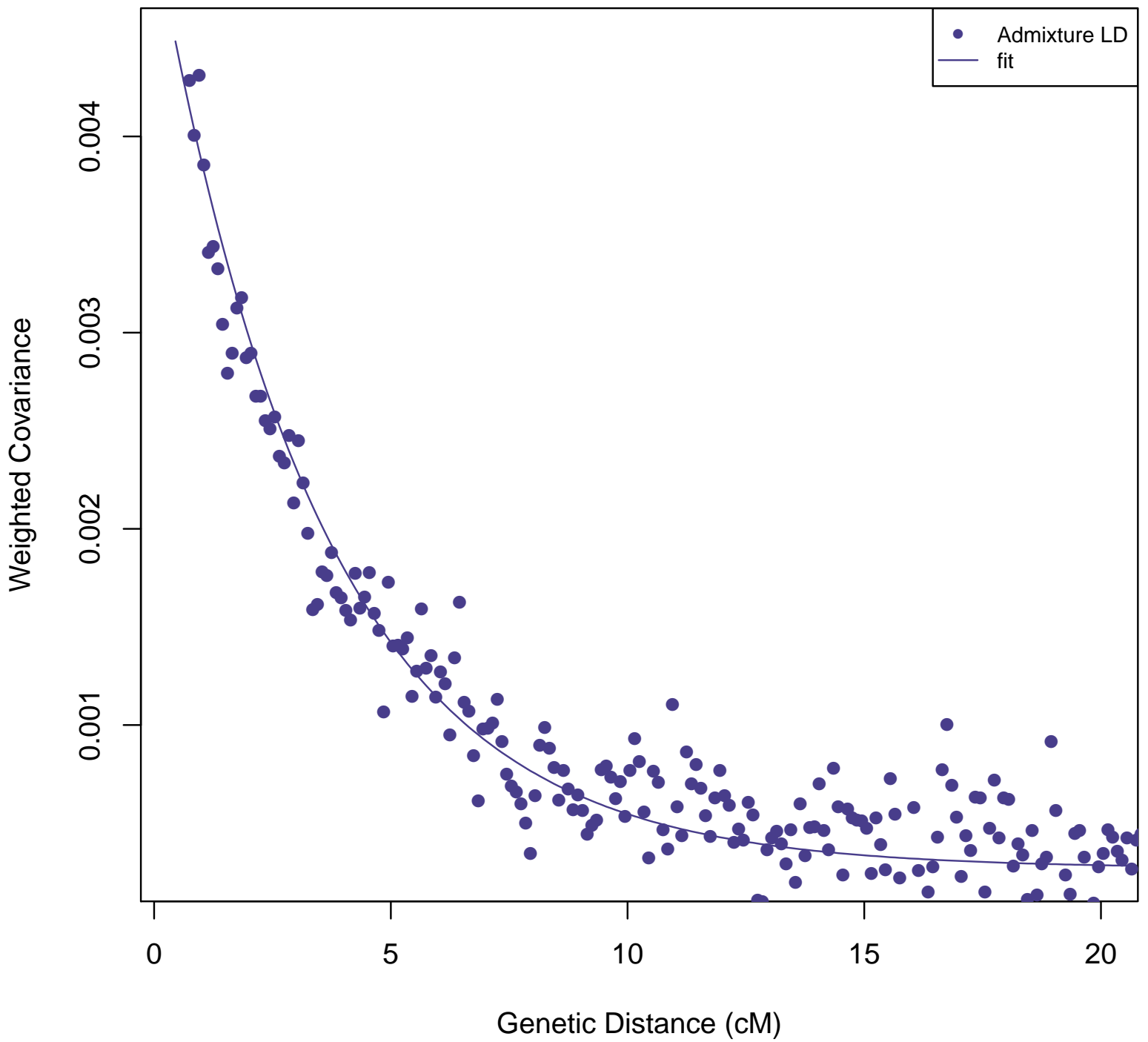
(b) ADMIXTURE Analysis



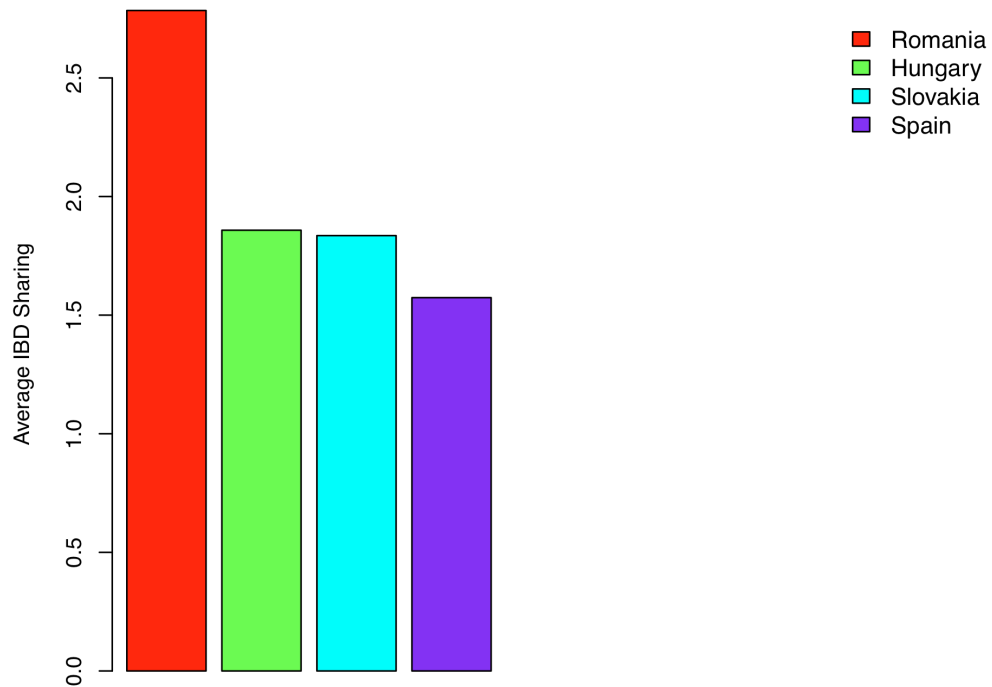
(c) PCA: Europeans, East Asians, South Asians and Roma



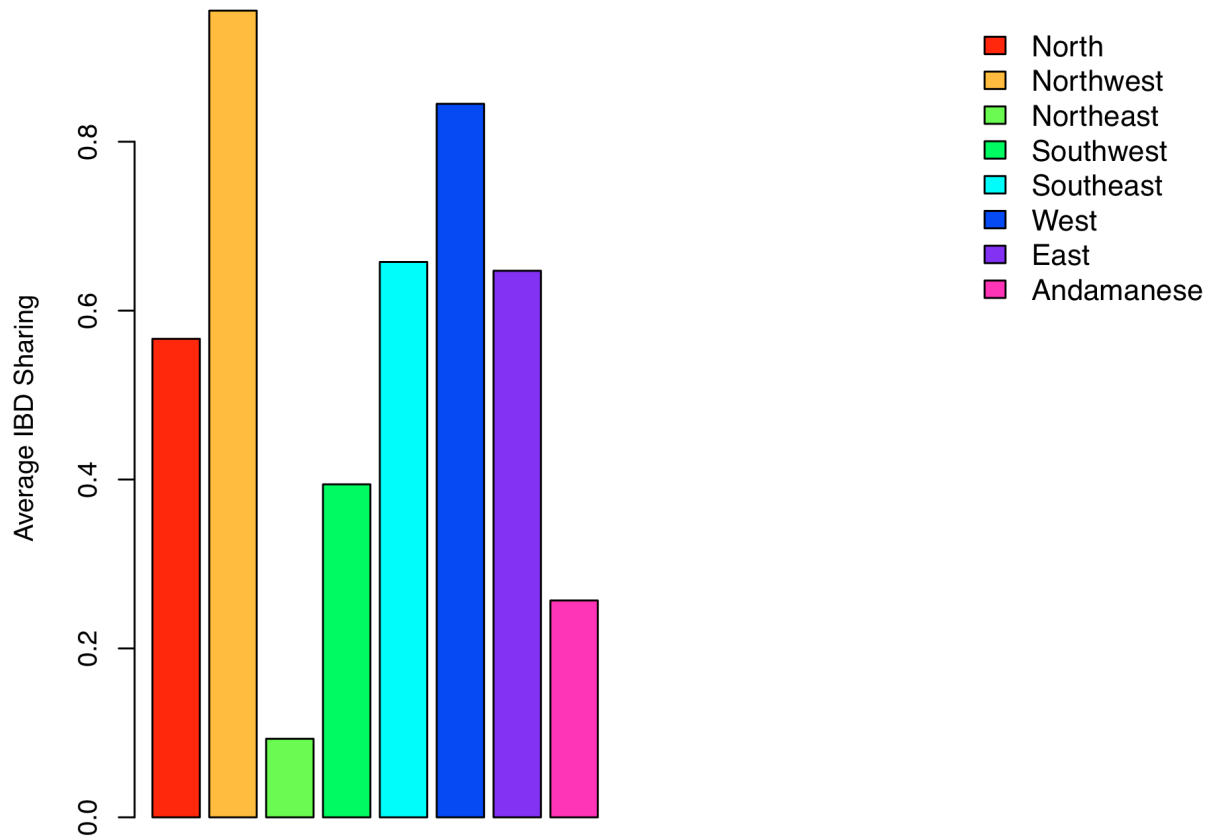
Rolloff Analysis: Roma



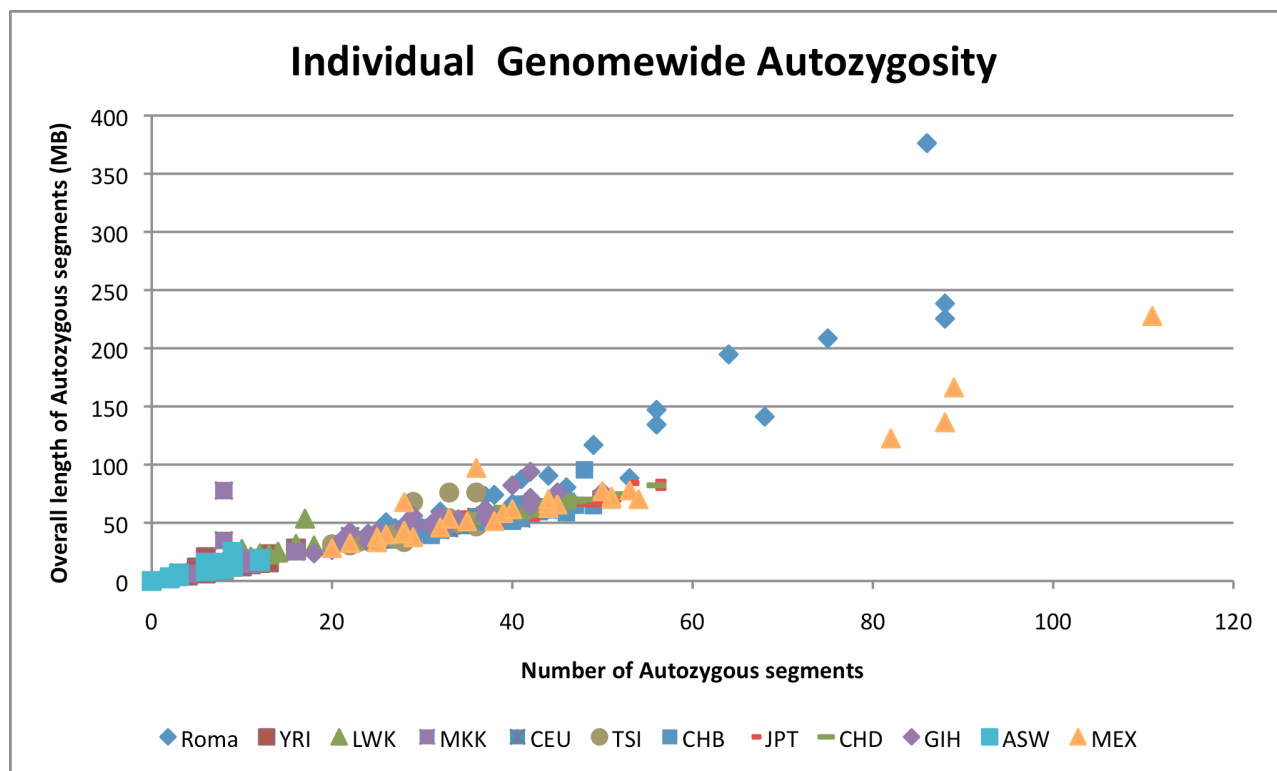
(a) Average pairwise IBD sharing with European hosts



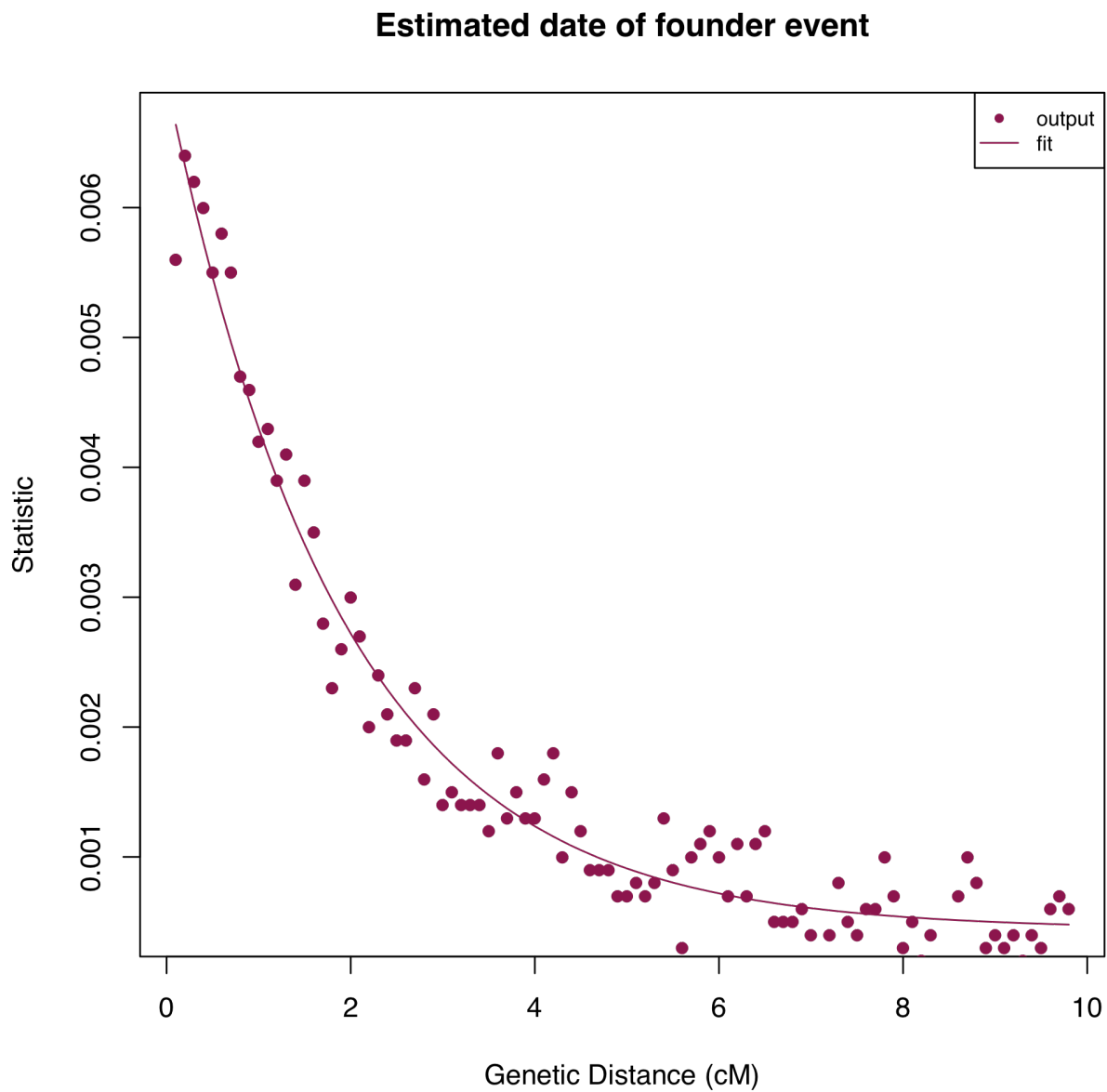
(b) Average pairwise IBD sharing with European hosts



(a)



(b)



Reconstructing Roma history from genome-wide data

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NOTE S1. New *ROLLOFF* statistic

In this note we consider alternative forms of the *ROLLOFF* linkage disequilibrium (LD) statistic[1] for dating population admixture events. We show that the original *ROLLOFF* statistic is susceptible to downward bias in the event of a recent population bottleneck, and we propose a modification of the statistic that is robust against such an effect (Table S3).

The *ROLLOFF* technique applies two key insights: first, that admixture creates LD that decays exponentially as recombination occurs—explicitly, as e^{-nd} , where n is the number of generations since admixture and d is the genetic distance between SNPs—and second, that the amount of admixture LD between each pair of SNPs is proportional to the product of the allele frequency divergences between the ancestral populations at those sites. The latter observation allows the e^{-nd} admixture LD decay signal to be detected (via a SNP-pair weighting scheme) and harnessed to infer the mixture date n .

The original *ROLLOFF* statistic captures admixture LD in the form of SNP auto-correlation. Defining $z(x, y)$ to be the (Fisher z -transformed) correlation coefficient between SNP calls at sites x and y , *ROLLOFF* computes the correlation coefficient between values of $z(x, y)$ and weights $w(x, y)$ over pairs of SNPs binned by genetic distance:

$$A(d) := \frac{\sum_{|x-y| \approx d} z(x, y) w(x, y)}{\sqrt{\sum_{|x-y| \approx d} z(x, y)^2} \sqrt{\sum_{|x-y| \approx d} w(x, y)^2}}, \quad (1)$$

the idea being that $A(d) \propto e^{-nd}$.

While this setup estimates accurate dates for typical admixture scenarios, it turns out to be noticeably biased in the case of a recent bottleneck. However, we will show that the following modified statistic does not suffer from the bias:

$$R(d) := \frac{\sum_{|x-y| \approx d} z(x, y) w(x, y)}{\sum_{|x-y| \approx d} w(x, y)^2}. \quad (2)$$

(Note that $R(d)$ amounts to taking the regression coefficient of $z(x, y)$ against the weights $w(x, y)$ for SNP pairs within each bin.)

An additional detail of our *ROLLOFF* variant is that we modify $z(x, y)$ to measure admixture LD as the covariance between SNPs x and y rather than the correlation (i.e., it equals the classical LD statistic D rather than the correlation r). We believe the use of covariance rather than correlation for $z(x, y)$ has little impact on the performance and properties of the statistic (as it roughly amounts to multiplying by a constant factor) but makes the statistic more amenable to mathematical analysis.

Explanation of bias from recent bottlenecks

The bias in the original formulation of *ROLLOFF* (1) introduced by a recent bottleneck can be readily explained at an intuitive level: the problem is that while the numerator of the correlation coefficient, $\sum_{|x-y|\approx d} z(x, y)w(x, y)$, decays as e^{-nd} as intended, the normalization term

$$\sqrt{\sum_{|x-y|\approx d} z(x, y)^2} \quad (3)$$

also exhibits a decay behavior that confounds the e^{-nd} signal (Figure S3). The reason is that a strong bottleneck introduces a very large amount of LD, effectively giving $z(x, y)$ a random large magnitude immediately post-bottleneck that is independent of the distance between x and y . This LD subsequently decays as e^{-nd} until the magnitude of $z(x, y)$ reaches the level of random sampling noise (arising from the finite sample of admixed individuals being used to calculate z). In non-bottlenecked cases, the square-norm of $z(x, y)$ is usually dominated by sampling noise, so the normalization term (3) effectively amounts to a constant, and dividing out by it has no effect on the decay rate of $A(d)$.

The “regression coefficient” version of the *ROLLOFF* statistic (2) does not contain the normalization term (3) and thus does not incur bias from bottlenecks.

Precise effect of genetic drift on original and modified *ROLLOFF* statistics

We now rigorously derive the above intuition. We will assume in the following calculations that the *ROLLOFF* weights are taken as the product of allele frequency divergences $\delta(x)$ and $\delta(y)$ in the ancestral mixing populations:

$$w(x, y) := \delta(x)\delta(y).$$

(Our reasoning below applies whether we have the true values of $\delta(x)$ and $\delta(y)$ or compute weights based on related reference populations or PCA loadings, however.) We also assume that all SNPs are polymorphic ancestrally—i.e., we ignore mutations that have arisen in the admixed population—and that the SNP ascertainment is unbiased with respect to the populations under consideration.

For a diploid population of size N with chromosomes indexed by $i = 1, \dots, 2N$, we set

$$z(x, y) := \frac{1}{2N} \sum_{i=1}^{2N} (X_i - \mu_x)(Y_i - \mu_y)$$

to be the covariance between binary alleles X_i and Y_i at sites x and y , respectively. We assume for ease of discussion that the data are phased; for unphased data, $z(x, y)$ is essentially a noisier version of the above because of cross terms.

We are primarily interested in the behavior of $z(x, y)$ from one generation to the next. Fix a pair of SNPs x and y at distance d and let z_0 denote the value of $z(x, y)$ at a certain point in time. After one generation, due to finite population size and recombination, the covariance becomes[2]

$$z_1 = z_0 e^{-d}(1 - 1/2N) + \epsilon, \tag{4}$$

where N is the population size, e^{-d} is the probability of no recombination, $(1 - 1/2N)$ is a Bessel correction, and ϵ is a noise term with mean 0 and variance on

the order of $1/N$. Iterating this equation over n generations, the final covariance is

$$z_n = z_0 e^{-nd} e^{-n/2N_e} + \epsilon_{\text{agg}},$$

where N_e is the effective population size over the interval and ϵ_{agg} is a sum of n partially decayed noise terms.

Now let time 0 denote the time of admixture between two ancestral populations mixing in proportions α and $\beta := 1 - \alpha$. (The bottleneck may have occurred either before or after this point, as long as it does not influence the calculation of the weights.) Then a little algebra shows that

$$E[z_0] = 2\alpha\beta\delta(x)\delta(y),$$

assuming the mixture is homogeneous and the distance d is large enough that background LD can be ignored. (In practice, heterogeneity in the admixed population changes the above form and results in the addition of an affine term to the *ROLLOFF* curve which we explicitly fit. We also typically fit only data from SNP pairs at distance $d > 0.5cM$ to avoid background LD.) We can now compute the modified *ROLLOFF* statistic:

$$\begin{aligned} E[R(d)] &= E \left[\frac{\sum_{|x-y|\approx d} z(x, y) \delta(x) \delta(y)}{\sum_{|x-y|\approx d} \delta(x)^2 \delta(y)^2} \right] \\ &\approx \frac{\sum_{|x-y|\approx d} [2\alpha\beta\delta(x)\delta(y)e^{-nd}e^{-n/2N_e} + \epsilon_{\text{agg}}] \delta(x)\delta(y)}{\sum_{|x-y|\approx d} \delta(x)^2 \delta(y)^2} \\ &\approx 2\alpha\beta e^{-nd} e^{-n/2N_e}. \end{aligned}$$

Importantly, in the last step we use the fact that the combined noise term ϵ_{agg} is uncorrelated with $\delta(x)\delta(y)$. Thus, even a strong bottleneck with a low value of N_e only scales $R(d)$ by the constant factor $e^{-n/2N_e}$, and the e^{-nd} scaling of the *ROLLOFF* curve as a function of d is unaffected.

On the other hand, if we use the original correlation form (1) of the *ROLLOFF* statistic $A(d)$, then the numerator still has the form of an exponential decay Ae^{-nd} ,

but now we divide this by the norm $\sqrt{\sum_{|x-y|\approx d} z(x,y)^2}$. In the case of a strong bottleneck, $z(x,y) = z_0 e^{-nd} e^{-n/2N_e} + \epsilon_{\text{agg}}$ can be dominated by the aggregate noise term ϵ_{agg} . Indeed, if the bottleneck occurred k generations ago, then the noise terms ϵ_i from the time of reduced population size will have decayed by e^{-kd} since the bottleneck but can still have large variance if the population size N_{bot} was very small at the time. In this case, at lower values of d , $E[z(x,y)^2] = E[(z_0 e^{-nd} e^{-n/2N_e} + \epsilon_{\text{agg}})^2]$ will be dominated by $E[\epsilon_{\text{agg}}^2]$ which will scale approximately as e^{-2kd}/N_{bot} . Hence, the denominator of $A(d)$ will be significantly larger at low d than at high d , causing a partial cancellation of the exponential decay of the *ROLLOFF* curve and thus a downward bias in the estimated date of admixture.

NOTE S2. Simulations for estimating dates of admixture events**Simulation 1: To test the effect of founder events post admixture**

In order to test the effect of founder events post admixture, we performed simulations using MaCS[3] coalescent simulator. We simulated data for three populations (say, A , B and C). We set the effective population size (N_e) for all populations to 12,500 (at all times except during the founder event), mutation and recombination rate to 2×10^{-8} and to 1×10^{-8} per base pair per generation respectively. C can be considered as an admixed population that has 60%/40% ancestry from A' and B' (admixture time (t) was set to 30/ 100 generations before present). A' and A diverged 120 generations ago, B' and B diverged 200 generations ago and A and B diverged 1800 generations ago. At generation x ($x < t$), C undergoes a severe founder event where the effective population size (N_e) reduces to 5 individuals for one generation. At generation ($x+1$), the $N_e = 12,500$. We simulate data for 5 replicates for each parameter. We performed *ROLLOFF* analysis (using the original and modified statistics) with C as the target and A and B as the reference populations. When we use the original *ROLLOFF* statistic, we observe that the dates are biased downward in cases of founder events post admixture. However, when we use the modified statistics, the bias is removed (Table S3). Details of the bias correction are shown in Note S1. Throughout the manuscript, we use the modified *ROLLOFF* statistic ($R(d)$) unless specified otherwise.

Simulation 2: To test the accuracy of the modified *ROLLOFF* statistic

We perform simulations using the same simulation framework as in reference [1] to test the accuracy of the estimated dates using the modified *ROLLOFF* statistic. We simulated data for 25 admixed individuals using Europeans (HapMap CEU) and HGDP East Asians (Han) as ancestral populations, where mixture occurred between 10-300 generations ago and European ancestry proportion was set to

20%. These ancestral populations were chosen as $F_{st}(\text{CEU}, \text{Han}) = 0.09$ is similar to the F_{st} between the ancestral populations of the Roma. Figure S4 shows that we get accurate estimates for the dates of mixture up to 300 generations.

Simulation 3: To test the effect of using PCA loadings instead of allele frequencies as weights in *ROLLOFF*

In the case of Roma admixture, data from unadmixed South Asian populations is not available and so it is not possible to compute the allele frequencies of SNPs for one ancestral population. However, data from many South Asian populations (which are admixed with ANI and ASI ancestry) are available and can be used for estimating the PCA-based SNP loadings. We simulations described below that mimic this scenario -

We simulated data for 60 admixed individuals using Europeans (HapMap CEU) and HGDP East Asians (Han) as ancestral populations, where mixture occurred 100 generations ago and European ancestry proportion was set to 30% (group 1: $n = 20$), 50% (group 2: $n = 20$) and 70% (group 3: $n = 20$). These three groups of simulated samples can be roughly considered as three South Asian populations. We performed PCA analysis with CEU and Groups 1-3 of simulated samples to estimate the SNP loadings that can be used in *ROLLOFF*.

Next, we simulated data for 54 individuals that can be used as the target in the *ROLLOFF* analysis. These individuals have 80%/20% European and East Asian ancestry respectively (similar to Roma) and the date of mixture is set to 30 ($n = 27$) and 100 ($n = 27$) generations before present. We ran modified *ROLLOFF* statistic to estimate the date of mixture in this panel of individuals using the PCA-based loadings computed above. We estimated that the dates of mixture were 33 ± 1 and 99 ± 1 generation for mixture that occurred 30 and 100 generations ago respectively (Figure S5). This shows that we can effectively estimate the date of mixture even in the absence of data from unadmixed ancestral populations, as

long as data from other admixed individuals (involving the relevant ancestral populations) is available.

Simulation 4: To test the model of two waves of admixture

In order to obtain an interpretation of the *ROLLOFF* estimated date of mixture when the model assumption of single wave of mixture is incorrect, we ran modified *ROLLOFF* statistic to infer the date of admixture on data simulated under a double admixture scenario. We simulated data using Europeans (HapMap CEU) and HGDP East Asians (Han) as the ancestral populations using the simulation framework described in reference [1]. We simulated double admixture scenarios in which a 50%/50% admixture of CEU and Han occurred at λ_1 (shown in Table S4), followed by a 60%/40% mixture of that admixed population and CEU at λ_2 (shown in Table S4). The mixture proportions were chosen so that the final European ancestry proportion is ~80% (similar to Roma). We ran modified *ROLLOFF* with a non-overlapping set of Europeans and Han as the reference population. Table S4 shows that as the interval ($\lambda_2 - \lambda_1$) between the multiple waves of mixture increases, the estimated dates of mixture reflects the date of the more recent gene flow event.

NOTE S3. Computing corrected IBD sharing distance between Roma and Indian groups.

To find the source of the Indian ancestry in Roma, we inferred the pairwise IBD sharing distance between Roma and various Indian groups. We observed that the Roma share the highest proportion of IBD sharing with groups from the northwest of India (Figure 3b). We were concerned that high IBD sharing could be an artifact related to the high proportion of ANI ancestry in the North-western Indian groups. Hence, we performed a regression analysis to correct for the effect of the ANI ancestry proportion on IBD sharing distance. The model that provided the best fit was $\text{IBD sharing} = 0.3558 + 0.8169 \times \text{ANI ancestry proportion}$ (P-value < 0.05). Each Indian group was considered as a single data point for this analysis. Next, we computed an average corrected IBD sharing measure for each region by regression out the effect of ANI ancestry and computing an average of the residuals for each region in India. Note: For this analysis, we did not include the Eastern Indian populations (Nyasha and Ao Naga) and Andamanese populations (Onge and Great Andamanese) as these populations do not have ANI ancestry.

In order to control for the effect of the sample size on the IBD computation, we performed bootstrap analysis such that for each run, we randomly sampled up to 30 individuals (some groups had < 30 samples) from each of the 8 Indian groups and estimated the IBD sharing statistics between Roma and the Indian groups. We performed a total of 100 runs and obtained the mean and standard error of the IBD statistic (Figure S7). We observed that Roma still share the highest proportion of IBD segments with groups from Northwest of India.

NOTE S4. Simulations for estimating date of founder event.

We used MaCS[3] coalescent simulator to perform simulations to test the robustness of our allele sharing statistic that we use for estimating the dates of the founder event. We simulated data for two populations (say, A and B) that diverged 1800 generations ago. We set the effective population size for both populations as $N_e = 12,500$, mutation rate = 2×10^{-8} and recombination rate = 1×10^{-8} per base pair per generation respectively. For each simulation, we compute the autocorrelation of allele sharing within B , and then subtract the across-population autocorrelation between A and B to remove the effects of ancestral allele sharing

Simulation 1: Founder event only

Pop B undergoes a severe founder event x generations ago where the effective population size reduces to 5 individuals for one generation. At generation $(x+1)$, the population size = N_e again. Table S5 shows that we can accurately estimate the date of the founder event using our statistic.

Simulation 2: Founder event and admixture

We simulate data for a more complex demography where B is admixed and has 40% ancestry from A' which is closely related to A . The admixture occurred at time t and at time $x = 10, 30$ or 100 generations, B undergoes a severe founder event where the effective population size of B reduces to 5 individuals for one generation. Table S5 shows that for a recent founder event (10 and 30 generations ago), we accurately estimate the date of the founder event. However, for older founder events (100 generations), we are unable to accurately estimate the date of the founder event, if it occurred pre-admixture. However, this is expected as we are only sampling the admixed population and not the ancestral population that underwent the founder event.

Simulation 3: No Founder event

We simulate data for a complex demography where B is admixed and has 40% ancestry from A' which is closely related to A . The admixture occurred either 10, 30, 50 or 70 generations ago. In all cases, we observe that the allele sharing statistic is not associated to distance. We test if the model of a straight line ($y \sim c$) or exponential decay ($y \sim c + Ae^{-tD}$), where D = genetic distance and t = time of founder event) is a better fit to the output. In all four cases, we fail to reject the null model ($y \sim c$) ($P > 0.05$).

Figure S1. ADMIXTURE Analysis of Roma and HapMap3 populations.

Results for ADMIXTURE analysis for K=2 to K=7. Each vertical line represents an individual colored in proportion to their estimated ancestry within each cluster.

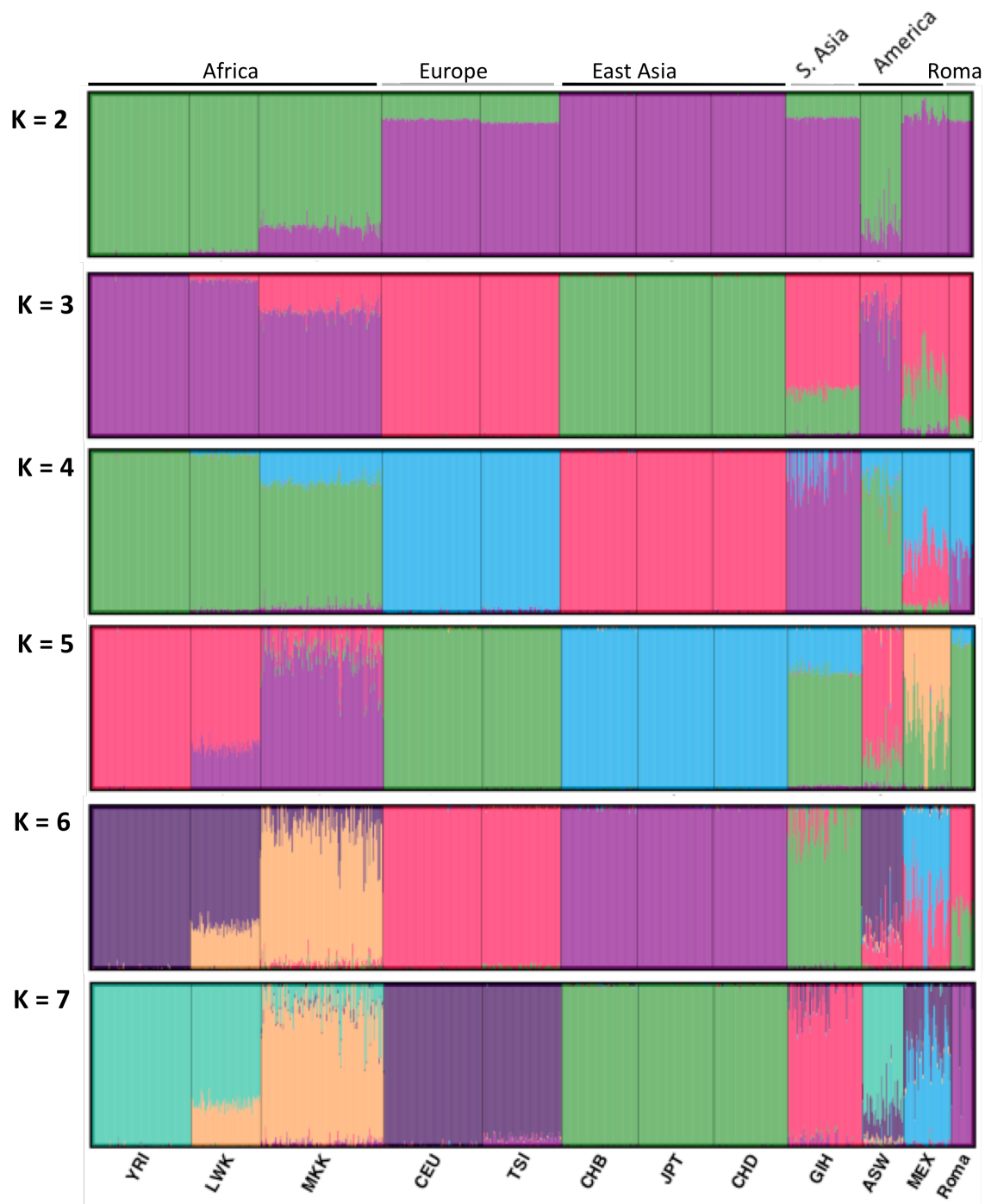


Figure S2. Estimating the proportion of Eurasian and South Asian ancestry in Roma. In order to estimate the proportion of West Eurasian ancestry in Roma, we use the phylogenetic tree shown below. The different colored lines show drift that has occurred between the populations connected by the line. The orange line shows the drift between YRI and Adygei (a population from the Caucasus) and the red and green lines shows the drift separating Roma and Onge. m denotes the shared drift between Roma and Onge. See methods for details for estimating the West Eurasian ancestry proportion (p) in Roma. This figure is adapted from reference [4].

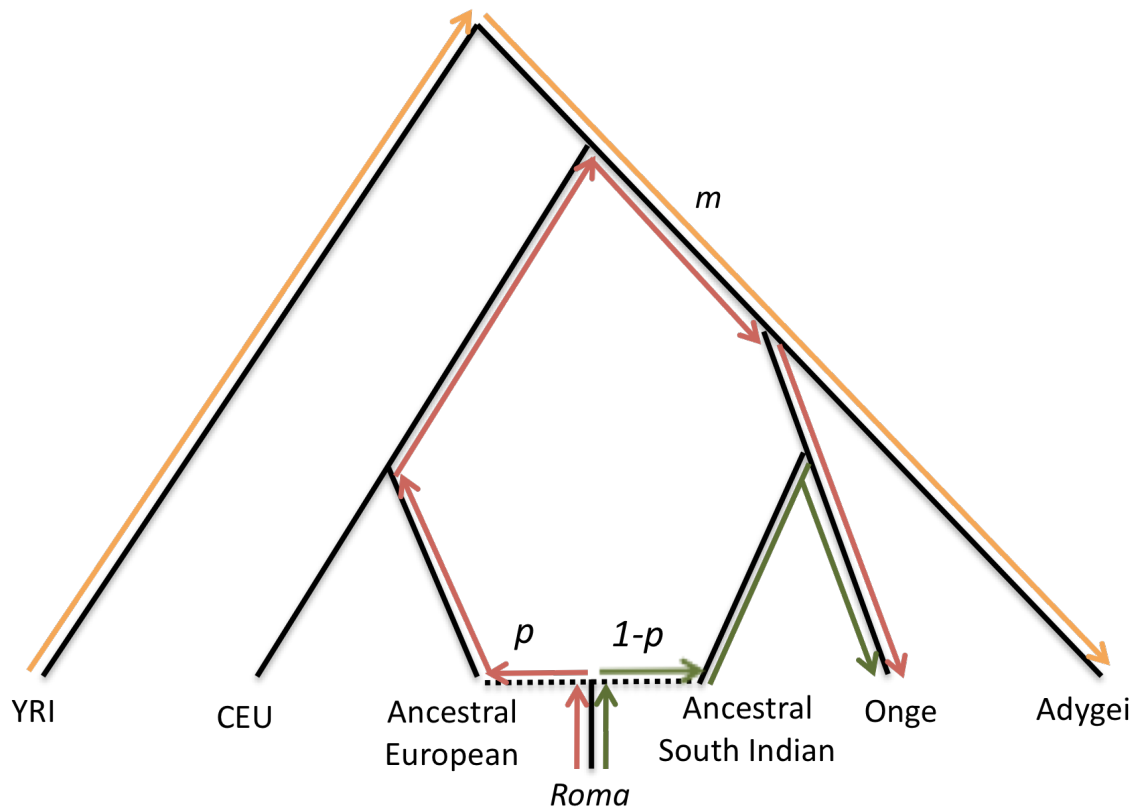
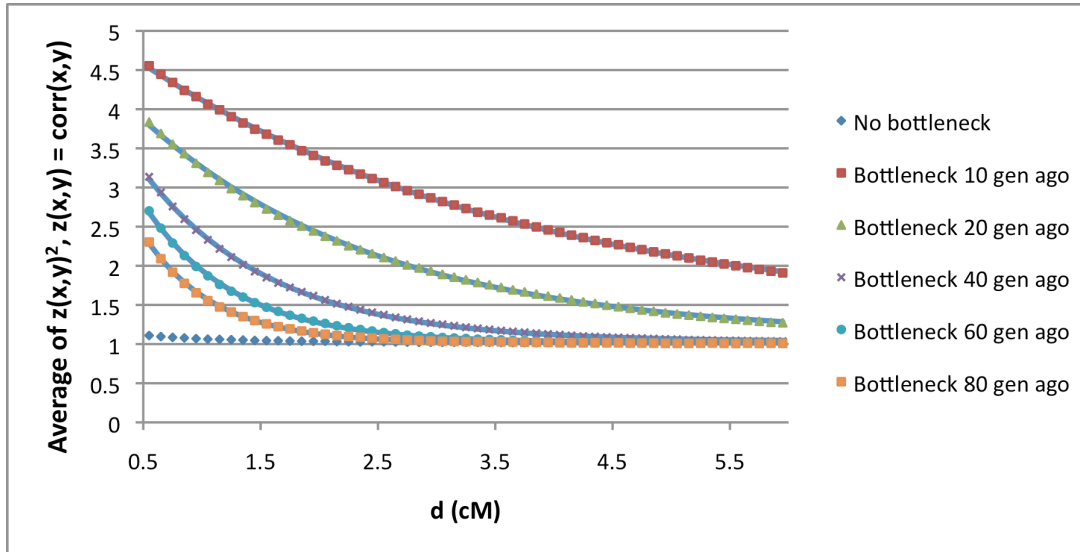


Figure S3. Normalization term from original *ROLLOFF* correlation coefficient formulation. We plot the squared normalization term $\sum_{|x-y|=d} z(x,y)^2$ as

a function of genetic distance d between SNPs for the admixture plus bottleneck scenarios described in Table S3, using either the correlation (a) or covariance (b) versions of $z(x,y)$. In the case of no bottleneck, the normalization term is dominated by finite sampling noise and exhibits no dependence on d . For the cases of a strong bottleneck post-admixture, however, $\sum_{|x-y|=d} z(x,y)^2$ exhibits an exponential decay $Ae^{-2kd} + c$ with rate constant approximately equal to twice the age of the bottleneck (best-fit $k = 15, 25, 46, 65, 83$ (a) and $k = 12, 20, 41, 60, 78$ (b) shown as solid lines).

(a) Using $z(x,y) = \text{correlation}(x,y)$



(b) Using $z(x,y) = \text{covariance}(x,y)$

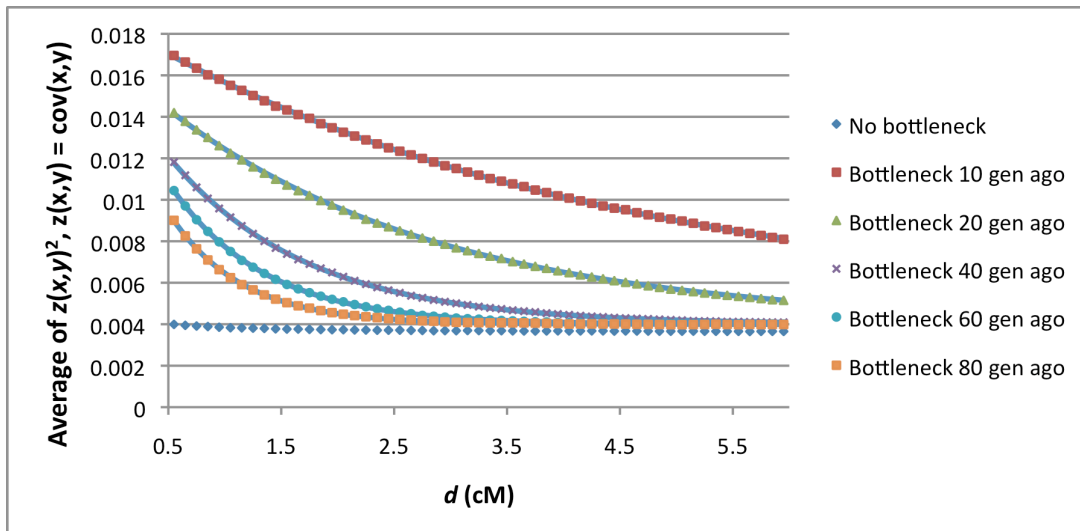
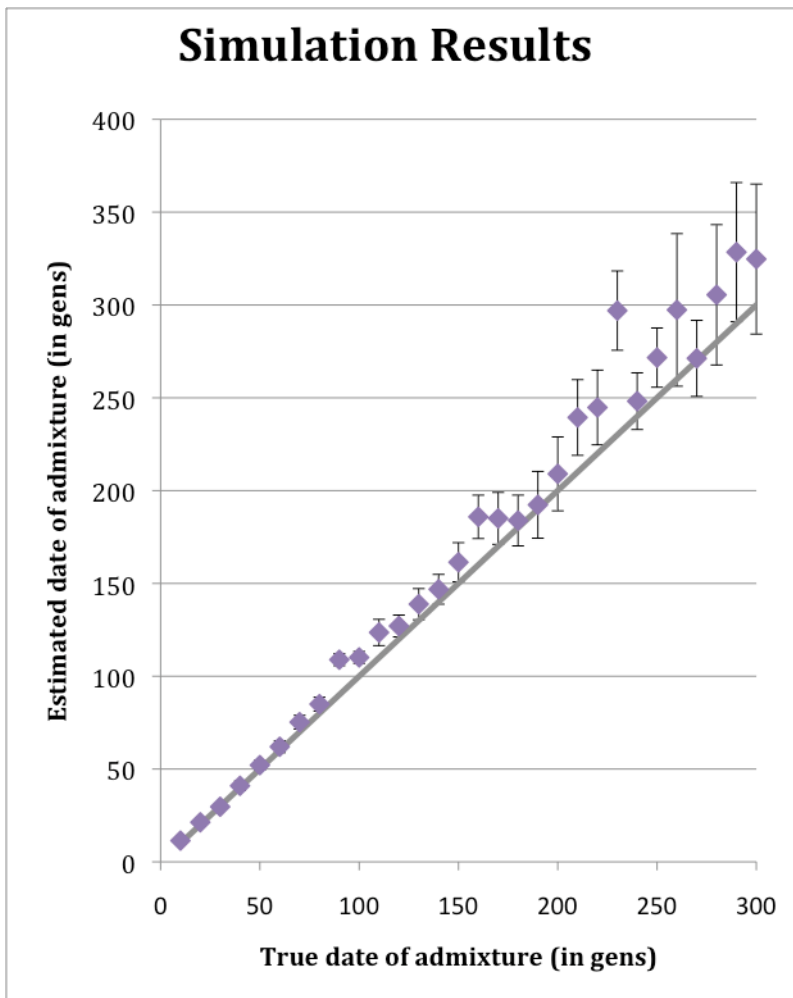


Figure S4. *ROLLOFF* Simulation Results: Variable age of mixture. We simulated data for 25 admixed individuals with mixed European and East Asian ancestry where the proportion of European ancestry was set to 20% and set the admixture date between 10-300 generations (as shown below). We ran the modified *ROLLOFF* statistic to estimate the date of mixture using allele frequencies in an independent dataset of French and East Asians. Standard errors were computed using weighted block jackknife as described in the Methods.



SUPPLEMENTARY MATERIAL

Figure S5. *ROLLOFF* Simulation using PCA-loadings. We simulated data for 54 individuals with mixed European and East Asian ancestry where the proportion of European ancestry was set to 80% (similar to Roma) and the mixture occurred 30 generations ago (left panel: $n = 27$) and 100 generations ago (right panel: $n = 27$). We ran *ROLLOFF* to estimate the date of mixture in this panel of individuals using the PCA-based loadings computed above. We estimated that the dates of mixture were 33 ± 1 and 99 ± 4 generations (the true dates were 30 and 100).

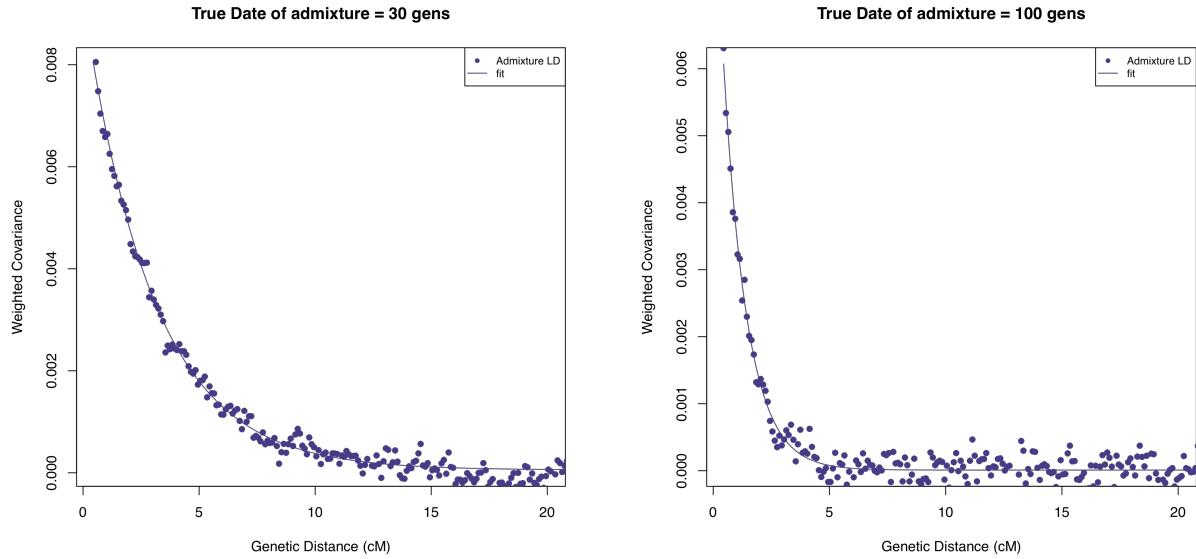


Figure S6. IBD Sharing of Roma with host European populations. We computed average pairwise IBD sharing between Roma from each geographical region and Europeans from that region (non-Roma European individuals from the countries in which the Roma were sampled).

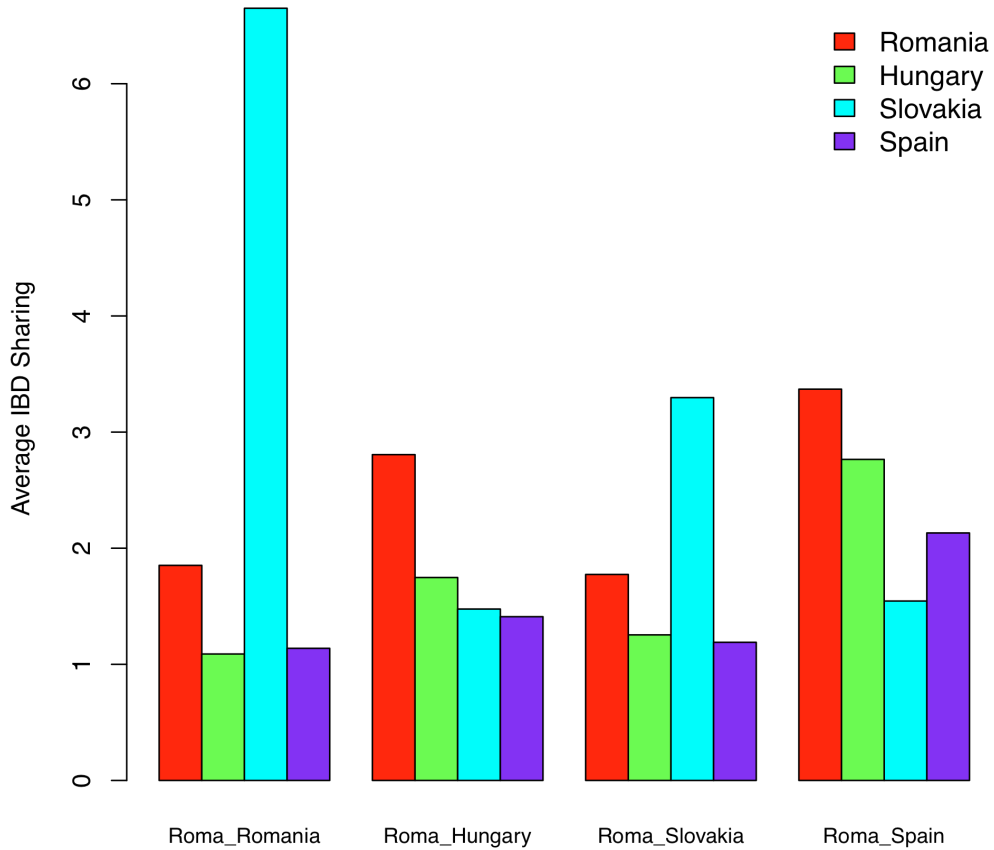
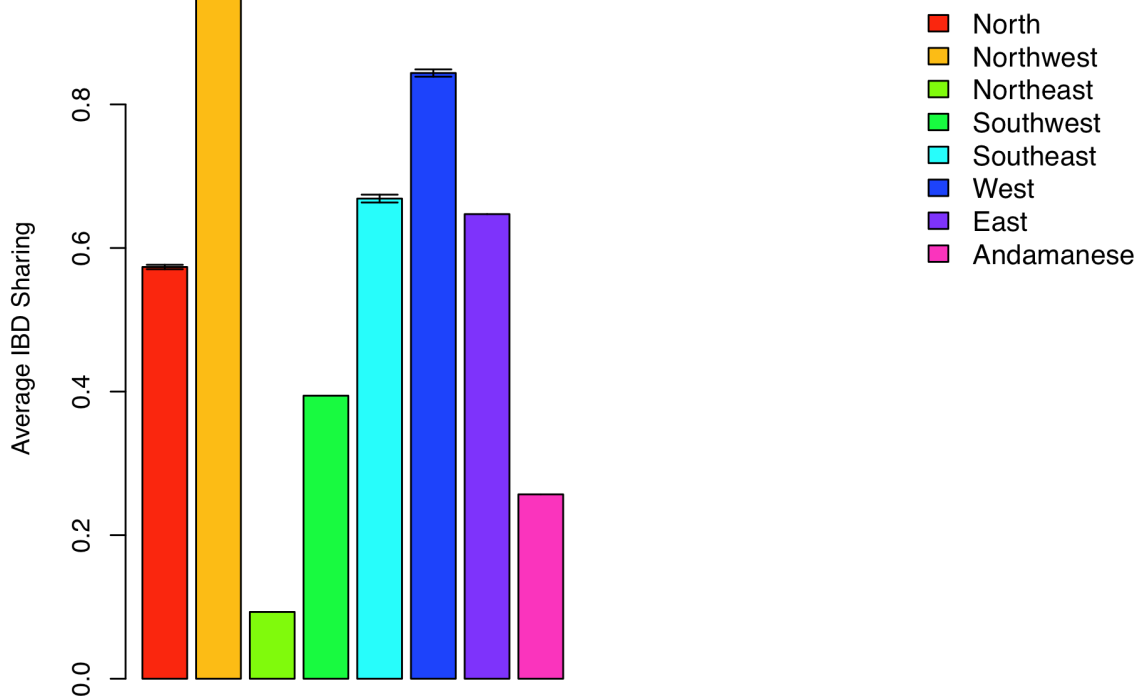


Figure S7. Bootstrap analysis to compute error in IBD statistics. We performed bootstrap analysis where we randomly sample up to 30 individuals from each of the 8 Indian groups and compute the IBD sharing statistics between Roma and the Indian groups. We performed a total of 100 runs and obtained the mean and standard error of the IBD statistic (vertical bars shown below). For Indian groups which had < 30 samples (such as Northeast, Southwest, East and Andamanese), all samples were included in each run and so no standard errors are shown.



SUPPLEMENTARY MATERIAL

Table S1. Average frequency differentiation (F_{st}) for Roma and HapMap populations

	CEU	YRI	CHB	JPT	ASW	CHD	GIH	LWK	MEX	MKK	TSI	Roma
CEU	0	0.14	0.102	0.104	0.088	0.103	0.033	0.13	0.036	0.093	0.003	0.016
YRI	0.14	0	0.169	0.17	0.008	0.169	0.129	0.007	0.134	0.025	0.136	0.135
CHB	0.102	0.169	0	0.007	0.127	0.001	0.071	0.159	0.064	0.131	0.102	0.092
JPT	0.104	0.17	0.007	0	0.129	0.008	0.072	0.161	0.065	0.133	0.104	0.094
ASW	0.088	0.008	0.127	0.129	0	0.128	0.083	0.009	0.088	0.013	0.086	0.087
CHD	0.103	0.169	0.001	0.008	0.128	0	0.071	0.16	0.066	0.132	0.103	0.093
GIH	0.033	0.129	0.071	0.072	0.083	0.071	0	0.119	0.038	0.086	0.032	0.026
LWK	0.13	0.007	0.159	0.161	0.009	0.16	0.119	0	0.125	0.015	0.126	0.125
MEX	0.036	0.134	0.064	0.065	0.088	0.066	0.038	0.125	0	0.093	0.037	0.04
MKK	0.093	0.025	0.131	0.133	0.013	0.132	0.086	0.015	0.093	0	0.088	0.089
TSI	0.003	0.136	0.102	0.104	0.086	0.103	0.032	0.126	0.037	0.088	0	0.015
Roma	0.016	0.135	0.092	0.094	0.087	0.093	0.026	0.125	0.04	0.089	0.015	0

Table S2. Formal tests of admixture

Population (X)	Sam- ples	Region	Z-score for 4 Population test			Estimated West Eurasian Ancestry %
			$\frac{(P_{CEU}-P_{YRI})}{\times (P_{Onge}-P_X)}$	$\frac{(P_{YRI}-P_{Onge})}{\times (P_{CEU}-P_X)}$	$\frac{(P_X-P_{YRI})}{\times (P_{CEU}-P_{Onge})}$	
Roma	18	Hungary	-33	4.8	-29.3	78.3 ± 1.9%
Roma*	3	Slovakia	-26.6	3.5	-22.8	71.5 ± 3.1%
Roma**	1	Romania	-20.2	0.7	-19.2	79.4 ± 4.7%
Roma	2	Spain	-25.3	0.9	-24	75.6 ± 4.0%
Roma	24	Combined	-33	4.8	-29.5	77.5 ± 1.8%

NOTE: * indicates that some samples from the group appear to have recent European gene flow. These samples were excluded from the analysis (the number of * indicates the number of samples excluded). Ancestry proportions were estimates based on f_4 Ratio Estimation using Yoruba, Adygei, Europeans (CEU) and Onge as the reference populations.

SUPPLEMENTARY MATERIAL

Table S3. Simulations for estimating dates of admixture events: Founder events post admixture model

True date of admixture	True date of founder event (x)	Date based on original <i>ROLLOFF</i> statistic (a)	Date based on modified <i>ROLLOFF</i> statistic (b)	Date based on modified <i>ROLLOFF</i> statistic (c)
30	N/A	31.3	32.0	32.1
30	5	24.6	30.1	29.0
30	10	27.7	34.1	32.3
30	20	23.3	32.7	31.0
30	25	23.4	30.8	29.5
100	N/A	94.1	96.8	97.0
100	10	93.9	106.1	102.9
100	20	87.1	102.7	97.3
100	40	75.3	95.6	92.2
100	60	83.9	106.3	102.8
100	100	81.6	101.1	99.0

Note: We simulated data from three populations Pop A (n = 20), Pop B (n = 20) and Pop C (n = 30) using MaCS coalescent simulator. Populations A and B diverged 1800 generations ago. The effective population size for all populations was set 12,500 at all times (except during the founder event). The mutation and recombination rates were set to 2×10^{-8} and 1×10^{-8} per base pair per generation. Pop C can be considered as an admixed population that has ancestry 60%/40% ancestry from A' and B' (admixture time (t) is set to 30/ 100 generations). Pop A' and A diverged 120 generations and B' and B diverged 200 generations ago. At generation x (shown in table above), Pop C undergoes a severe founder event where the effective population size reduces to 5 individuals for one generation. When x = N/A, there was no founder event. We performed *ROLLOFF* (using original and modified statistic) with Pop C as the target and Pop A and B as the reference populations. We performed 5 replicates for each parameter and report the average estimated date of mixture. The statistics used were -

(a) Original *ROLLOFF* Statistic: $A(d) = \frac{\sum_{|x-y|=d} z(x,y)w(x,y)}{\sqrt{\sum_{|x-y|=d} z(x,y)^2} \sqrt{\sum_{|x-y|=d} w(x,y)^2}}$; where $z(x,y)$ = correlation between x and y.

(b) Modified Statistic: $R(d) = \frac{\sum_{|x-y|=d} z(x,y)w(x,y)}{\sum_{|x-y|=d} w(x,y)^2}$; where $z(x,y)$ = correlation between x and y.

(c) Modified Statistic: $R(d) = \frac{\sum_{|x-y|=d} z(x,y)w(x,y)}{\sum_{|x-y|=d} w(x,y)^2}$; where $z(x,y)$ = **covariance** between x and y.

Table S4. Simulations for estimating dates of admixture events: Two gene flow model

Date of first wave of mixture (λ_1)	Date of second wave of mixture (λ_2)	Estimated date in generations (\pm standard error)
120	20	36 \pm 3
170	20	28 \pm 2
220	20	23 \pm 2
270	20	24 \pm 2
320	20	25 \pm 1
370	20	25 \pm 1
420	20	22 \pm 1
130	30	46 \pm 3
180	30	47 \pm 3
230	30	41 \pm 2
280	30	39 \pm 2
330	30	39 \pm 3
380	30	35 \pm 2
430	30	32 \pm 3

Note: We simulated 27 individuals using CEU and Han Chinese as the ancestral populations where we set the overall European ancestry proportion to be 80%. We then performed *ROLLOFF* analysis using the modified statistic with an independent dataset of Europeans (HGDP French) and East Asians (HapMap CHB) as reference populations.

Table S5. Simulations for estimating dates of founder events

Simulation scenario	True date of founder event	True date of admixture	Estimated date of founder event (in generations)
<u>Founder event only</u>			
	10	--	11.2
	20	--	20.8
	40	--	39.3
	60	--	52.7
	80	--	74.9
	100	--	95.7
<u>Founder event + Admixture</u>			
	10	10	8.2
	10	20	8.4
	10	40	8.3
	10	60	9.2
	10	80	11.8
	10	100	9.9
	30	10	24.4
	30	20	29.9
	30	30	30.1
	30	40	26.5
	30	60	26.2
	30	80	27.9
	30	100	27.6
	100	10	50
	100	20	60.9
	100	40	67.4
	100	60	81.5
	100	80	113.3
	100	100	92.7
	100	150	85.3

Note: We simulated 20 individuals from Pop A and 25 individuals from Pop B using MaCS coalescent simulator. The two populations diverged 1800 generations ago. The effective population size for both populations was set 12,500 at all times (except during the founder event). The mutation and recombination rates were set to 2×10^{-8} and 1×10^{-8} per base pair per generation. During the founder event, the effective population size reduced to 5 individuals for one generation at the date specified in the table above. For each simulation we generated data for ~450,000 polymorphic sites. SNPs with minor allele frequencies of <1% were discarded.

References

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